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Central pharmacological activity of a new piperazine derivative: 4-(1-Phenyl-1h-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester

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ABSTRACT

Aims: Our study focuses on the design and synthesis of a new piperazinic derivate, 4-(1-phenyl-1h-Pyrazol-4-Ylmethyl)-Piperazine-1-Carboxylic Acid Ethyl ester (LQFM008), and evaluation of its anxiolytic-like profile in *Swiss* mice.
Main methods: LQFM008 was evaluated in a screening test of the central nervous system including the rota-rod, sodium pentobarbital-induced sleep, open field, elevated plus maze and light–dark box tests.
Key findings: LQFM008 induced convulsions at the dose of 1.1 mmol/kg (i.p., s.c. or p.o.). LQFM008 up to 400 μmol/kg had no effect in the rota rod test. In the open field test, LQFM008 increased the number of crossings and the time spent at the central area as well as the sleeping time in sodium pentobarbital-induced sleep. In the elevated plus maze and light–dark box tests, this compound showed an anxiolytic-like activity. This anxiolytic-like activity was antagonized by NAN-190 (5-HT_{1A} antagonist) but not by flumazenil (benzodiazepine antagonist).
Significance: The compound LQFM008 showed anxiolytic-like activity which may involve serotonergic pathway.

Introduction

Anxiety is a normal psychological and physiological state characterized by somatic, emotional, cognitive and behavioral components, being necessary for the survival of animals as well as human beings. However, when that feeling of anticipation present at levels higher than those considered normal, the anxiety becomes pathological and has to be treated (Schmitt, 2003).

The drugs most used in the treatment of generalized anxiety are the benzodiazepine, a positive modulator of GABA transmission, and the buspirone, an anxio-selective drug which is a partial agonist of serotoninergic receptor type 1A (5-HT_{1A}) (Andreatini et al., 2001; Clement and Chapouthier, 1998; Katzman, 2011).

Buspirone is a piperazine derivative; piperazine compounds constitute a very large chemical class and some of these compounds have the ability to cross the blood–brain barrier due to the small size and the lipophilic nature of their molecules. Thus, some piperazine derivatives promote activity upon the central nervous system and are used in the treatment of various mental disorders, including anxiety disorders, Alzheimer's disease, psychosis and depression (Katzman, 2011; Menegatti et al., 2003; Papakostas and Fava, 2007; Sadashiva et al., 2006).

In order to explore the central pharmacological activity of the piperazine derivatives, as well as to perform a quicker synthesis and to obtain simpler compounds, this paper proposes the design, synthesis and evaluation of the central pharmacological activity of the new piperazine derivative 4-(1-phenyl-1H-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester (LQFM008) in mouse behavioral models.

Material and methods

Chemical synthesis

The synthetic route planned to achieve the new piperazinic derivate LQFM008 (7) (Fig. 1) consists of three stages: first, the 1-phenyl-1H-pyrazole (3) was synthesized through the classical method described by Finar and Hurlock (1957), but the chemoselective and regiospecific formylations of 1-phenyl-1H-pyrazoles were performed under Duff's conditions (Johansson et al., 2003; Lalehzari

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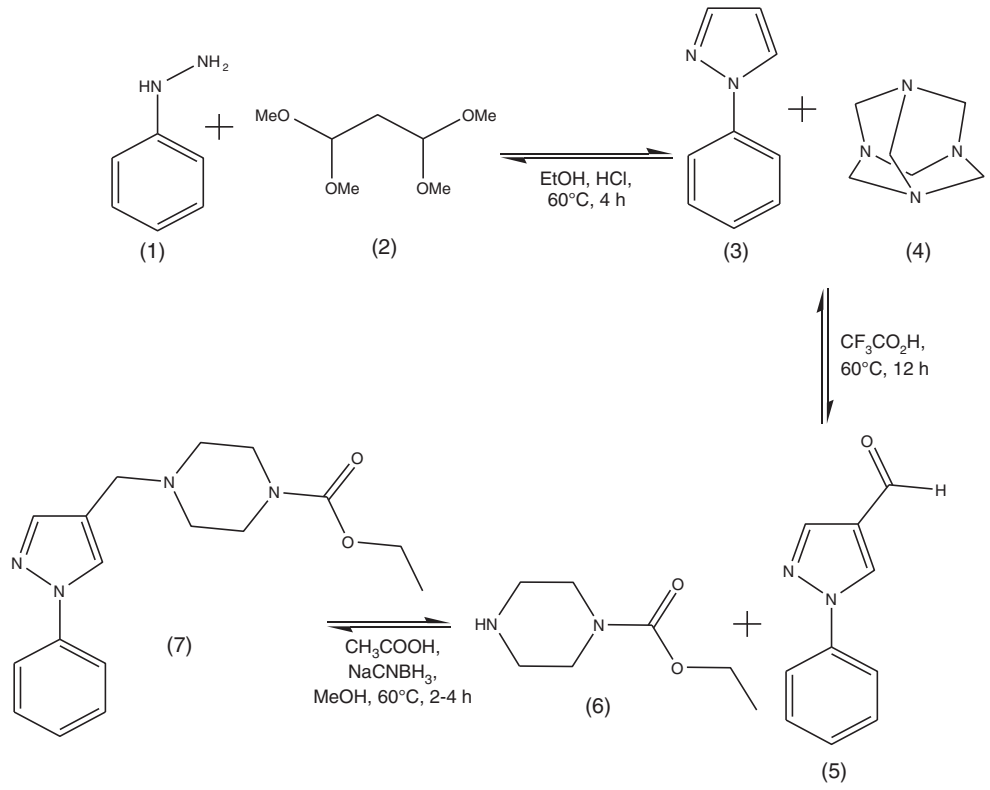

Fig. 1. The synthetic route planned to achieve the new piperazinic derivate 4-(1-phenyl-1H-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester (LQFM008; 7).

et al., 2008; Lindoy et al., 1998). The key step of the synthesis consisted of the reductive amination of formyl phenyl pirazole (5) to obtain the 4-(1-phenyl-1H-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester (LQFM008; 7); this step was performed under reflux for 2–4 h with 0.7 mmol of phenyl pirazole (5), 0.7 mL of ethyl N-piperazine carboxylate (6), 7 drops of acetic acid, 4.07 mmol of $NaCNBH_3$ and 2.5 mL of methanol (Luo et al., 2004).

The melting points were determined with resonance (^{1}H NMR) and were determined in deuterated chloroform containing a Gehaka PF1500®. Proton magnetic ca. 1.0% tetramethylsilane as internal standard in a Bruker Avance® 500. Carbon magnetic resonance (^{13}C NMR) was determined with the same spectrometer described above at 125 MHz, using deuterated chloroform containing ca. 1.0% tetramethylsilane. Infrared (IR) spectra were obtained with a Perkin-Elmer Spectrum BXII FT-IR® spectrophotometer using potassium bromide plates.

The progress of all reactions was monitored by TLC performed on aluminum sheets which are precoated with silica gel 60 (F 254, Whatman®) to a thickness of 0.2 mm. The mobile phase was n-hexane: ethyl acetate (70:30). The developed chromatograms were viewed under ultraviolet light at 254 nm.

1-Phenyl-1H-pyrazole (3)

Reaction condensation between phenyl hydrazine (1) and 1,1,3,3, tetrametoxypropane (2) in 98% yield, as red oil, Rf = 0.48 (n-hexane: ethyl acetate, 70:30). IR (liquid film) cm^{-1}: 3447–3124 (νC–H); 1598–1494 (νC═C and C═N); ^{1}H NMR (500 MHz) CDCl3/TMS (δ): 7.73 (dd, J = 1.9 and 0.5 Hz, H-3); 6.47 (dd, J = 2.4 and 1.9 Hz, H-4); 7.93 (dd, 2.4 and 0.5 Hz, H-5); 7.69 (dddd, 7.5, 2.2, 1.6 and 0.7 Hz, H-2′); 7.45 (dddd, 7.5, 7.4, 1.9 and 0.9 Hz, H-3′); 7.29 (tdd, 7.4,1.6 and 1.2 Hz, H-4′); 7.45 (ddd, 7.4, 7.2, 1.9 and 0.7 Hz, H-5′) and 7.69 (ddd, 7.2, 2.2, 1.2 and 0.9 Hz, H-6′). ^{13}C NMR (125 MHz) CDCl3/TMS (δ): 141.1 (C-3); 107.7 (C-4); 126.6 (C-5); 140.1 (C-1′); 119.2 (C-2′); 129.3 (C-3′); 126.4 (C-4′); 129.3 (C-5′) and 119.2 (C-6′).

1-Phenyl-1H-pyrazole-4-carbaldeyde (5)

Derivate (5) was obtained in 58.28% yield, as a brown solid, mp 61–68 °C, Rf = 0.72 (n-hexane:ethyl acetate, 70:30). IR (KBr) cm^{-1}: 3126 (νC–H); 1681 (νC═O); 1546–1507 (νC═C and C═N); ^{1}H NMR (500 MHz) CDCl3/TMS (δ): 8.17 (d, 0.5 Hz, H-3); 8.44 (d, 0.5 Hz, H-5); 7.73 (ddd, 7.4, 2.1 and 1.2 Hz, H-2′); 7.51 (dddd, 7.4, 7.0, 2.0 and 1.9 Hz, H-3′); 7.39 (tt, 7.0 and 1.2 Hz, H-4′); 7.51 (ddd, 8.2, 7.0 and 1.9 Hz, H-5′); 7.73 (dddd, 8.2, 2.1, 2.0 and 1.2 Hz, H-6′) and 9.97 (s, CHO). ^{13}C NMR (125 MHz) CDCl3/TMS (δ): 141.7 (C-3); 125.6 (C-4); 130.0 (C-5); 139.0 (C-1′); 119.8 (C-2′); 129.8 (C-3′); 127.9 (C-4′), 129.8 (C-5′); 119.8 (C-6′) and 184.2 (CHO).

4-(1-Phenyl-1H-pyrazole-4yl methyl)-piperazine-1-carboxylic acid ethyl ester (LQFM008, 7)

Reductive amination between phenyl pirazole (5) and ethyl N-piperazine carboxylate (6) in 55% yield, as yellow solid, mp 98–100 °C (n-hexane:ethyl acetate, 70:30). IR (KBr) cm^{-1}: 3115 (νC–H); 1505–1340 (νC═C and C═N); 1607 (νC═O); 1270 (νC═O); ^{1}H NMR (500 MHz) CDCl3/TMS (δ): 7.87 (1H, s, H-5); 7.67 (2 H, dd, J = 1.4 and J = 8.8 Hz, H-2′ and 6′); 7.64 (1H, s, H-3); 7.46–7.43 (2 H, dd, J = 7.6 and J = 8.0 Hz, H-3′ and 5′); 7.29–7.26 (1H, H-4′); 4.12 (2 H, dd, J = 7.0 Hz, H-6); 3.05 (2 H, s, H-14); 3.52–3.47 (4 H, m, H-9 and 11); 2.47–2.41 (4 H, m, H-8 e 12); 1.25 (3 H, J = 7.0 Hz, H-15); ^{13}C NMR (125 MHz) CDCl3/TMS (δ): 155.3 (1C, C-13); 141.9 (1C, C-3); 140.1 (1C, C-1′); 129.4 (2C, C-3′ and 5′); 126.4 (1C, C-5′); 126.2 (1C, C-4′); 119.0 (1C, C-4); 118.4 (2C, C-6′ and 2′); 61.1 (1C, C-14); 52.4 (1C, C-6); 52.2 (2C, C-8 and 12); 43.4 (2C, C-9 and 11); and 29.6 (1C, C-15).

Pharmacological evaluation

Drugs

Diazepam (Diazepan®, Cristália, Brazil), sodium pentobarbital (Sigma Chemical Co., USA), polisobarte 80 (Tween 80 — Synth, EUA), flumazenil (União Química, Brazil), buspirone (Libbs, Brazil),

hydrobromide 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)-butyl]-piperazine (NAN-190 — Chemical Co., USA) were used in the present study. It is well known that benzodiazepines act as anxiolytic-like at low doses, and they also produce sedation and myorelaxant effect at higher doses (Novas et al. 1988), thereby, our group has used diazepam 3.51 μmol/kg (1 mg/kg) as the standard drug for anxiolytic-like effect, and diazepam 17.51 μmol/kg (5 mg/kg) as the standard drug for sedative and myorelaxant effects.

Animals

Adult male *Swiss* mice weighing approximately 30 g were used in all experiments. The animals were provided by the Central Animal House of Federal University of Goiás (Universidade Federal de Goiás — UFG); they were housed in groups of 20 mice/cage and were kept in a room with controlled temperature (25 ± 1 °C) and lighting (light/dark cycle of 12 h, light on at 7 am), with food and water ad libitum. The animals were kept in the laboratory for an adaptation period of at least 1 h before the experiments. The tests are usually conducted between 9:00 a.m. to 3:00 p.m. All experiments were carried out in accordance with the principles of ethics and animal welfare recommended by the Brazilian Law (# 11.794 — 10/08/2008) and the experimental protocols were approved by the Ethics Commission of the UFG (no. 104/11).

Effects on gross behavior or Irwin test

This test was used to assess preliminary drug effects on mice behavior and toxicity, and also to determine effective dose range. Experimental groups of mice (n = 3) were treated orally (p.o.), intraperitoneally (i.p.) or subcutaneously (s.c.) with LQFM008 at the doses of 1.1 mmol/kg, 0.3 mmol/kg and 0.06 mmol/kg, whereas control groups received vehicle (2% Tween-80, 10 mL/kg) by the same routes. Animals were observed in free ambulation on a flat surface for 3 min, at 0, 5, 10, 20, 30 and 60 min, 4, 8, 24 and 48 h, 4 and 7 days after the treatment. The presence or absence of mortality, seizure, erection of the tail (Straub sign), sedation, excitation, motor incoordination, abdominal torsion and spontaneous ambulation was recorded. The observed effects were compiled using a standard pharmacological screening approach, adapted from that described by Malone (1977).

Rota rod test

It is a test that permits the detection of potential neurotoxic and/or muscle relaxing agents. Animals were trained previously (24 h before experiment) based on their capacity to maintain their balance (for 2 min) on the rotating bar (12 rpm) with an axis of 3 cm in diameter. For testing, the pre-trained animals (n = 9/group) were treated (p.o.) with vehicle (2% Tween-80, 10 mL/kg), LQFM008 at the doses of 100, 200 and 400 μmol/kg, or diazepam 17.51 μmol/kg. The mice were placed on the rota-rod after 60 min of treatments and the number of falls for up to 1 min and/or three falls were recorded (Dunham and Miya, 1957).

Sodium pentobarbital-induced sleep test

It is a useful test for detecting agents with sedative effects. Groups of mice (n = 9/group) were pre-treated with vehicle (2% Tween-80, 10 mL/kg, p.o.), LQFM008 at the doses of 100, 200 and 400 μmol/kg p.o., or diazepam 17.51 μmol/kg p.o. Sixty minutes after the treatment, the animals received sodium pentobarbital (221.0 μmol/kg, i.p.). Latency to induce sleep (loss of the righting reflex) and the duration of the sleeping time (the time required to recover the righting reflex) were recorded for each animal (Carlini and Burgos, 1979).

Open field test

It is a useful test to assess mice ambulatory behavior and also detect anxiolytic-like or anxiogenic-like agents. Sixty minutes after the treatment (n = 9) with vehicle (2% Tween-80, 10 mL/kg, p.o.), LQFM008 at doses of 100, 200 and 400 μmol/kg p.o., diazepam 3.51 and 17.51 μmol/kg p.o., the animals were placed at the center of an open field arena. The apparatus consisted of a circular arena measuring 36 (diameter) × 20 cm (height), with the bottom divided into eight squares of equal area. The number of squares crossed, number of rearing behaviors, immobility time (s), number of crossings and time (s) spent at the center of the arena were recorded in a 5 min session (Archer, 1973; Siegel, 1946).

Elevated plus maze test

It is a test validated for the detection of potentially effective anxiolytic-like or anxiogenic-like agents. Experimental groups of 9 mice were treated (p.o.) with vehicle (2% Tween 80, 10 mL/kg), LQFM008 (25, 50, 100, 200 or 400 μmol/kg) or diazepam (3.51 μmol/kg). The acrylic plus maze apparatus consisted of two open arms (30 × 5 cm) and two closed arms of the same size but with end and side walls measuring 25 cm in height, the arms were connected by a central 5 × 5 cm area, purchased from Insight Scientific Equipments — Brazil (model EP 150®). Sixty minutes after the treatment, animals were placed individually at the center of the elevated plus maze with their nose facing the direction of one of the enclosed arms, and observed for 5 min (Lister, 1987; Pellow et al., 1985). The test was carried out under a red light (15 W) and was fully recorded for later analysis. Parameters such as the time spent in the central platform, the number of entries and the time spent in the open and enclosed arms of elevated plus maze were used as a measure of anxiety. Anxiolytic compounds reduce the animal's aversion to the open arms and promote its exploration.

Light–dark box test

This test is one of the most frequently employed models for the assessment of the anxiolytic-like or anxiogenic-like activity of a novel agent. Sixty minutes after the treatment with vehicle (2% Tween 80, 10 mL/kg, p.o.), LQFM008 (25, 50, 100, 200 or 400 μmol/kg, p.o.) or diazepam (3.51 μmol/kg, p.o.), the animals (n = 9/group) were placed at the center of the light area (20 × 26.5 × 26 cm) facing the opening (7 × 7 cm) of the dark area (20 × 26.5 × 17.5 cm); the number of transitions between the two compartments and the time spent in the light area were recorded over a 5 min period (Crawley and Goodwin, 1980).

Influence of the time of treatment with LQFM008 in experimental tests of anxiety

Animals were evaluated in the elevated plus maze and in the light–dark box, 15, 30, 60 and 90 min after the treatment with LQFM008 200 μmol/kg p.o.

Investigation of the putative underlying mechanisms involved in the anxiolytic-like effect

The animals were pre-treated i.p. (n = 9) with saline 0.9%, NAN-190 1.3 μmol/kg (5-HT_{1A} antagonist) and flumazenil 6.6 μmol/kg (benzodiazepine antagonist). After 30 min, the animals were treated (p.o.) with vehicle (Tween 2.0%, 10 mL/kg), LQFM008 100 μmol/kg, diazepam 3.51 μmol/kg p.o. and buspirone 26 μmol/kg p.o. After 60 min, they were submitted to the elevated plus maze test.

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). Data was analyzed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls as the post-hoc test. For data without normal distribution, analysis was performed using Kruskal–Wallis's test followed by Dunn's test. Effects were considered significant at $p \leq 0.05$ (Sokal and Rohlf, 1981).

Results

Effects on gross behavior or Irwin test

LQFM008 at dose of 1.1 mmol/kg (p.o., i.p. or s.c.) induced generalized convulsions 5 min after treatments. However, LQFM008 at the doses of 0.3 and 0.06 mmol/kg (p.o., i.p. or s.c.) apparently decreased the spontaneous ambulation 5 min after the treatments. Based on these results, we opted for LQFM008 at the doses of 100, 200 and 400 μmol/kg to assess the subsequent pharmacological activities.

Rota rod test

The treatments with LQFM008 do not alter the number of falls as compared to the control group (Table 1) and, as expected, the treatment with diazepam 17.51 μmol/kg increased this parameter.

Sodium pentobarbital-induced sleep test

Treatment with LQFM008 100, 200 and 400 μmol/kg increased the sleeping time (Fig. 2A), without any alteration significant in the sleeping latency (Fig. 2B). Diazepam 17.51 μmol/kg increased the sleeping time and decreased the sleeping latency (Fig. 2).

Effect of LQFM008 in the experimental tests of anxiety

Open field test

The treatments with LQFM008 had no effects on total crossing, immobility time and rearing behavior, while the doses of 200 and 400 μmol/kg increase the time spent at the center and all the three doses increase the crossings in the central area (Table 1). As expected, diazepam 3.51 μmol/kg increases the time and crossings at the central area, while the dose of 17.51 μmol/kg increased the immobility time and decreased the total crossing and rearing behaviors.

Elevated plus maze test

Treatment with LQFM008, at the doses of 50, 100 and 200 μmol/kg, increased the entries and time spent in open arms, 400 μmol/kg increased only the number of entries; all doses of LQFM008 decreased the time spent at the central platform (Fig. 3). Diazepam 3.51 μmol/kg increased the entries and the time spent in open arms and decreased the time spent in the central platform (Fig. 3).

Light–dark box test

Treatment with LQFM008 100 and 200 μmol/kg increased the transitions and time spent in the light area while LQFM008 400 μmol/kg increased only the time spent in the light area (Fig. 4). Diazepam 3.51 μmol/kg increases these parameters (Fig. 4).

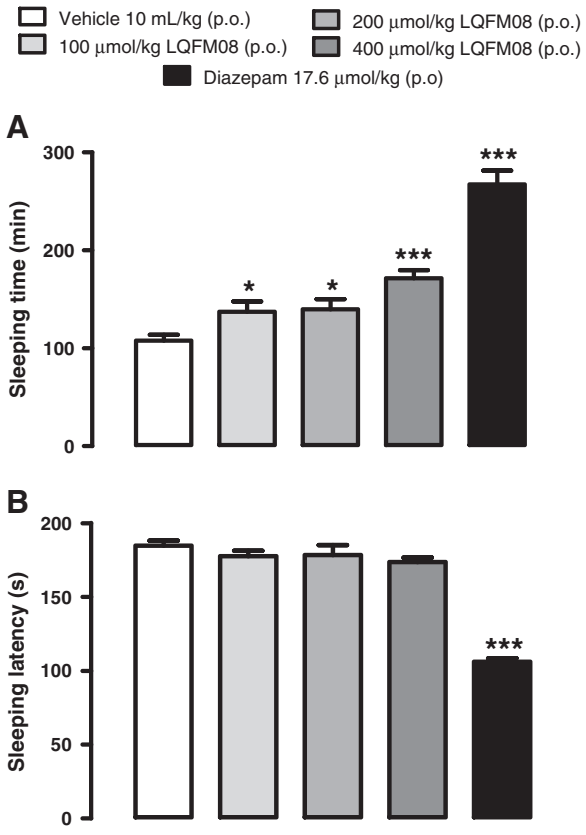

Fig. 2. Effect of LQFM008, at different doses (p.o.), in sodium pentobarbital-induced sleep test as evaluated in mice, on (A) latency to (s) and (B) on duration of sleeping time (min), compared with the control group (vehicle). ***$p \leq 0.001$ compared with the control group (one-way ANOVA followed by Student–Newman–Keuls' test).

Influence of the time of treatment with LQFM008 in two experimental tests of anxiety

LQFM008 200 μmol/kg showed an anxiolytic-like profile, in the elevated plus maze and light–dark box tests, only 60 and 90 min after the treatment (Table 2).

Table 1
Effects of the LQFM008 in the open field and rota-rod tests in mice.

	Vehicle	LQFM008			Diazepam	
	10 mL/kg	100 μmol/kg	200 μmol/kg	400 μmol/kg	3.51 μmol/kg	17.51 μmol/kg
Open field test						
Crossings	82.88 ± 6.05	90.83 ± 5.46	91.00 ± 4.13	83.14 ± 8.10	90.52 ± 8.06	52.00 ± 12.84**
Immobility	19.56 ± 4.12	16.17 ± 6.40	17.78 ± 3.21	13.67 ± 1.74	15.25 ± 3.65	166.50 ± 23.91***
Rearings	47.25 ± 3.81	48.00 ± 5.30	49.00 ± 3.35	46.62 ± 4.33	49.25 ± 4.69	11.90 ± 3.57***
%CrCe	43.41 ± 1.26	54.42 ± 2.21*	54.19 ± 2.60*	55.81 ± 1.16*	57.69 ± 1.58**	46.35 ± 4.80
TCe	78.62 ± 8.75	100.75 ± 7.30	116.14 ± 9.64*	114.00 ± 11.77*	130.02 ± 10.68**	70.83 ± 6.26
Rota rod test						
Number of falls	0.6 ± 0.16	0.3 ± 0.15	0.3 ± 0.15	0.4 ± 0.16	0.6 ± 0.70	2.3 ± 0.37*

Data are expressed as mean ± SEM, and were analyzed statistically using one-way ANOVA, followed by Student–Newman–Keuls as post-test. In the rota rod test, the data are expressed as mean ± SEM and were analyzed statistically using Kruskal–Wallis test, followed by Dunn's test as post-test. Number of crossings in the center of the open field — %CrCe, time spent in the center of the open field — TCe.

** $p \leq 0.01$.
*** $p \leq 0.001$.
* $p \leq 0.05$.

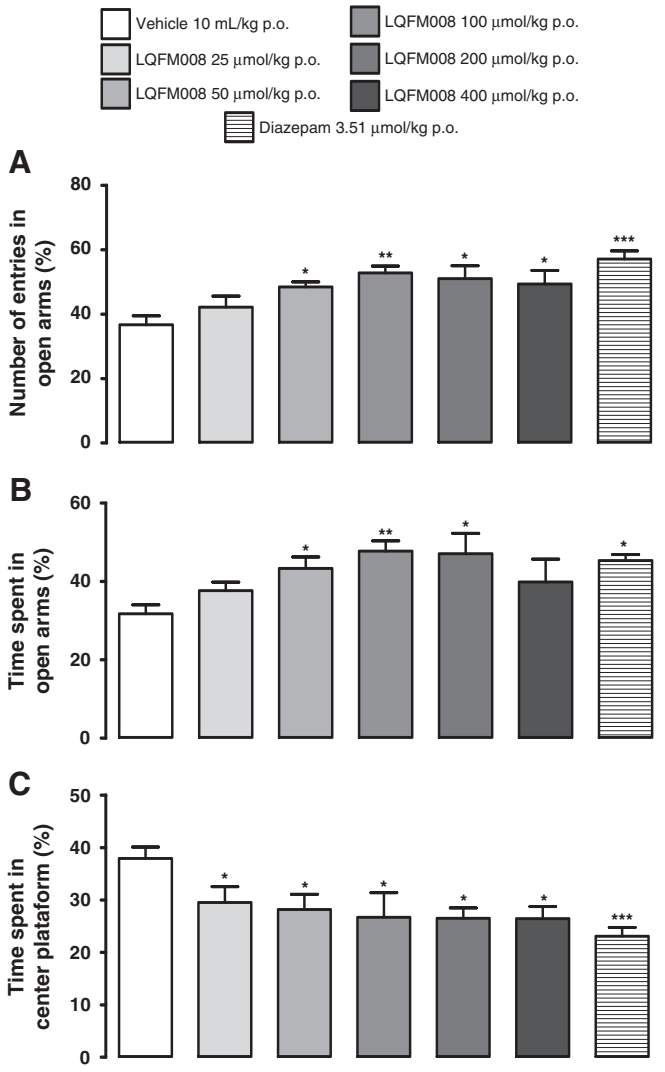

Fig. 3. Effects of LQFM008, at different doses (p.o.), on the elevated plus maze test, 60 min after treatments. (A) Number of entries in the open arms (%), (B) time spent in the open arms (%), and (C) time spent in the central platform (%). *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$, compared with the control group (one-way ANOVA followed by Student–Newman–Keuls' test).

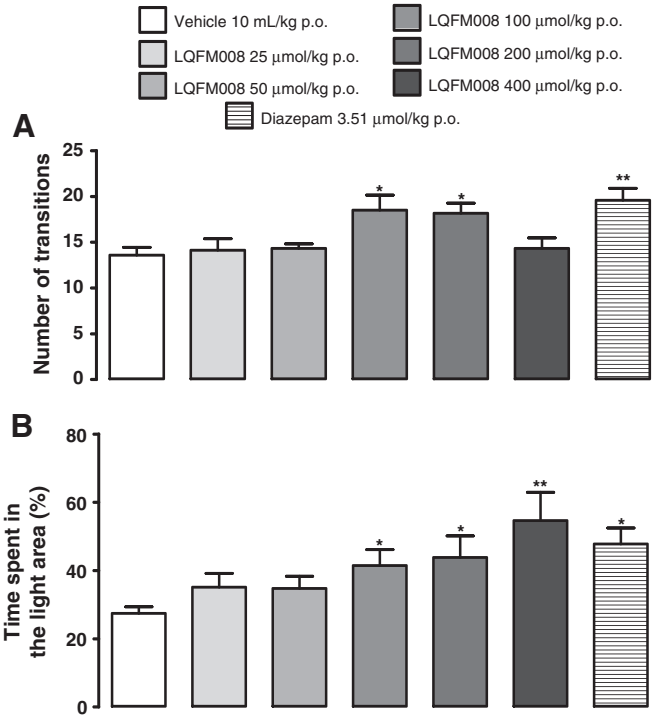

Fig. 4. Effects of LQFM008, at different doses (p.o.), on the light–dark box, 60 min after treatments. (A) Number of transitions between light and dark areas and (B) time spent in the light area (%). *$p \leq 0.05$ and **$p \leq 0.01$, compared with the control group (one-way ANOVA followed by Student-Newman-Keuls' test).

Investigation of the putative underlying mechanisms involved in the LQFM008 anxiolytic-like effect

The anxiolytic-like effect was not significantly decreased when flumazenil was injected before oral administration of LQFM008 100 μmol/kg. However, this effect was significantly decreased when the mice were pretreated with NAN-190. Flumazenil and NAN-190 per se did not show an effect but they antagonized anxiolytic effects of diazepam and buspirone, respectively (Fig. 5).

Discussion

A novel piperazine derivative (LQFM008), with putative central activities, was designed from clozapine by molecular simplification strategy. Considering the investigation of a new candidate to neuroactive drugs, the initial parameters investigated were the effects on gross behavior or Irwin test. Our results showed that the LQFM008 induced generalized convulsions at the dose of 1.1 mmol/kg.

The treatments with this compound did not alter the number of falls evaluated in the rota-rod; thus suggesting their non-interference with motor coordination of the mice. This result is consistent with the previous research on three phenylpiperazine derivatives by Neves et al. (2010) who reported absence of alteration in motor coordination of the experimental subject in the rota rod.

The sodium pentobarbital-induced sleep allows observation of putative depressant and/or stimulant activity of a given drug as a result of synergism or antagonism to the depressant activity of sodium pentobarbital. Depressant drugs reduce the latency and increase the sleeping time, whereas the reverse holds true for stimulant drugs (Carlini et al., 1986; Farkas et al., 2005). LQFM008, at different doses, increased the duration of sleep induced by sodium pentobarbital without reducing the latency, which could suggest a central depressant activity.

In order to investigate a specific central anxiolytic-like activity, open field, elevated plus maze and light–dark box tests were performed.

The open field test is used to assess the mice ambulatory behavior and may also detect anxiolytic-like or anxiogenic-like agents (Prut and Belzung, 2003). Animals treated with LQFM008, did not show any change in the total crossings, the immobility time and the number of rearing behavior, thereby suggesting no alteration in motor coordination. Hence, in respect of these results, the apparent reduction of spontaneous locomotion, seen in Irwin test, may be false-positive. Meanwhile, the LQFM008 increase in preference for central area of the open-field both in the number of crossing and the time spent suggests an anxiolytic-like activity. Meanwhile, different activities of piperazine derivatives have been reported in the open field test. Angrini et al. (1998) demonstrated that animals treated with buspirone did not alter the preference for the center of open-field, but increased the immobility time, while Rex et al. (1998) showed that locomotor activity of animals remained unaltered with ipsapirone treatment.

The elevated plus maze test is the most used test for the selection of anxiolytic-like drug prototype (Han et al., 2009; Avgustinovich et al., 2003). This test is an animal model that uses natural stimuli such as fear of new, open and high spaces (Yu et al., 2007) as well as the preference for enclosed spaces. LQFM008 increased the

Table 2
Effects of the compound LQFM008 200 μmol/kg on the elevated plus maze test and light–dark box tests, 15, 30, 60 and 90 min after treatments (p.o.) in mice.

	Vehicle	LQFM008 200 μmol/kg (p.o.)			
	10 mL/kg (p.o.)	15 min	30 min	60 min	90 min
Elevated plus maze test					
Entries in open arms (%)	30.19 ± 5.6	35.87 ± 3.3	40.67 ± 2.7	49.27 ± 3.3*	52.19 ± 6.2*
Time spent in open arms (%)	30.86 ± 5.3	28.17 ± 2.6	33.78 ± 5.2	53.20 ± 5.7*	52.25 ± 8.5*
Time spent in central platform (%)	42.30 ± 3.4	30.46 ± 4.2	30.50 ± 3.4	18.90 ± 3.2**	23.06 ± 4.1**
Light–dark box					
Transitions number	6.71 ± 1.0	10.43 ± 0.6	10.00 ± 1.3	15.00 ± 1.1***	18.50 ± 2.1***
Time spent on light area (%)	13.50 ± 1.6	17.27 ± 3.0	19.78 ± 4.1	36.93 ± 2.7***	42.67 ± 2.4***

Data are expressed as mean ± SEM. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001, compared with the control group (one-way ANOVA followed by Student-Newman-Keuls' test).

number of entries and the time spent in the open arms, and decreased the time spent in the central platform, corroborating the anxiolytic-like effect earlier observed in the open field.

In the light–dark box test, the anxiolytic-like activity is characterized by decrease in aversion for light area, increase in exploratory activity, increase in the time spent in the light area and in the number of transitions between both sides of the box (Chen et al., 2006; Hoog, 1996). In this test, LQFM008 also showed an anxiolytic-like effect by increasing the transitions and the time spent in the light area as compared with the control group.

The anxiolytic-like activity of LQFM008 became evident at 60 and 90 min after the treatment. Interestingly, there was no notable disparity between the treatment times in respect of the anxiolytic effect observed. Hence, 60 min was chosen for the investigation of

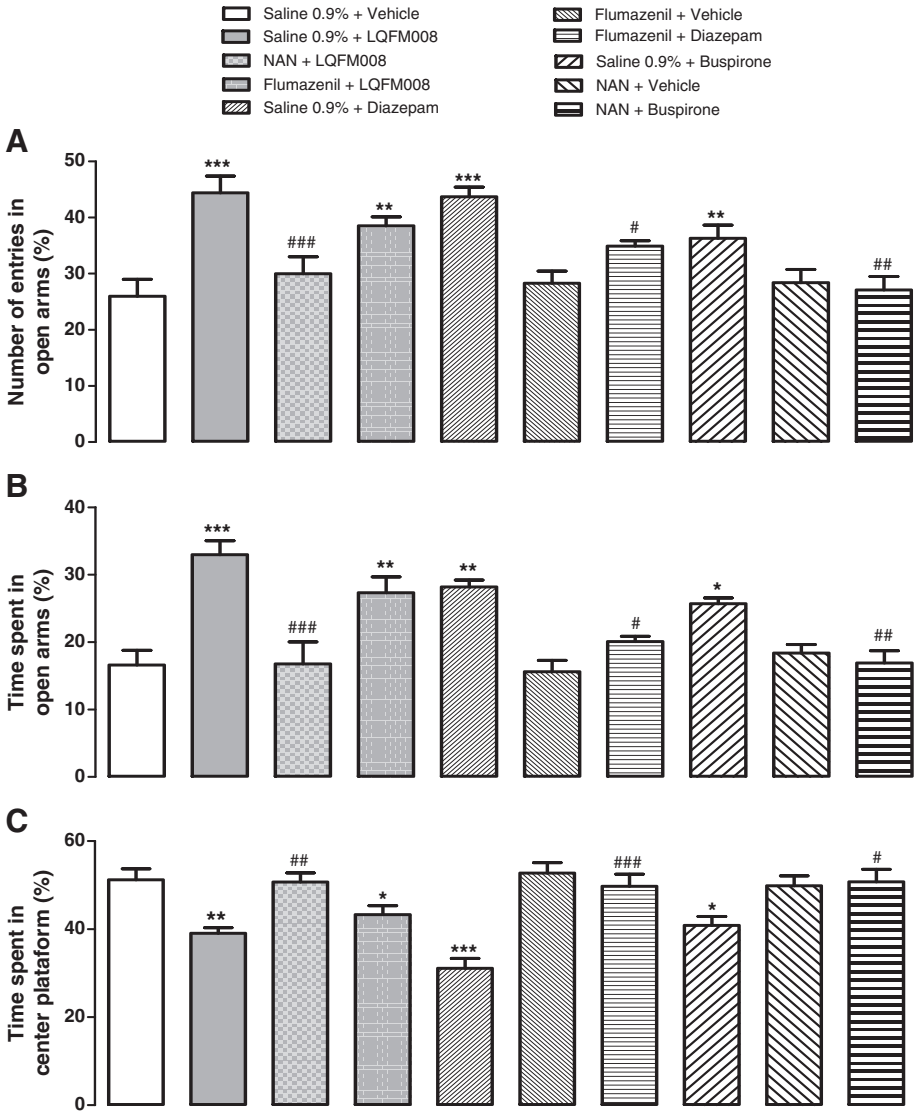

Fig. 5. Effects of the pre-treatment with NAN-190 1.3 μmol/kg i.p. and flumazenil 6.6 μmol/kg i.p. on the effects of LQFM008 100 μmol/kg p.o., diazepam 3.51 μmol/kg p.o. and buspirone 26 μmol/kg p.o. in the elevated plus maze. (A) Number of entries into the open arms, (B) time spent in the open arms (%), and (C) time spent in the central platform (%). *p ≤ 0.05, **p ≤ 0.01, ***p < 0.001, compared with the control group, and ##p < 0.01, ###p < 0.001, compared with the group treated with saline i.p. (vehicle) and LQFM008 100 μmol/kg p.o., saline i.p. (vehicle) and diazepam 3.51 μmol/kg p.o. or saline i.p. (vehicle) and buspirone 26 μmol/kg p.o. (one-way ANOVA followed by Student–Newman–Keuls' test).

putative underlying mechanism of action involved in LQFM008 anxiolytic-like activity.

In the study of mechanism of action, we proposed to investigate the involvement of the benzodiazepine site and the serotonin receptor type 1A. Pre-treatment with flumazenil (benzodiazepine antagonist) did not antagonize the LQFM008 anxiolytic-like activity in the elevated plus maze test, hence, excluding the benzodiazepine site in the LQFM008 effect. The pre-treatment with NAN-190 (a 5-HT_{1A} antagonist) antagonized the LQFM008 anxiolytic-like effect, when compared with the results observed after the treatment with LQFM008, thus, suggesting the participation of 5-HT_{1A} receptor in LQFM008 activity.

Although clinically the anxiolytic activity of 5HT_{1A} agonists that act on the somatodendritic autoreceptors in the dorsal raphe nucleus of midbrain raphe is a consequence of their desensitization during repeated treatment over several days. Meanwhile, in rodent experimental models, this anxiolytic activity can be observed after an acute treatment, as observed in this study and, by Cao and Rodgers (1997), Young and Johnson (1991) and Peng et al. (2004) that demonstrated the anxiolytic-like activity of buspirone, a 5-HT_{1A} partial agonist, in the elevated plus maze test and light–dark box tests after an acute treatment. Moreover, several reports in the literature have associated anxiolytic-like activity of piperazine derivatives to the involvement of serotoninergic pathway (Avgustinovich et al., 2003; Bojarski et al., 2006; Gray et al., 2009; Khatri et al., 2009; Paluchowska et al., 2007).

This result as a matter of fact does not necessarily exclude the involvement of other binding site of GABA and other neurotransmitters in LQFM008 anxiolytic-like effect. Future research will be targeted at elucidating the interaction of this compound with monoamine reuptake and metabolism.

Conclusions

The compound LQFM008 is a new piperazine derivative that has an anxiolytic-like activity in different animal models without compromising motor activity, and this activity seems to involve the participation of 5-HT_{1A} receptors.

Conflict of interest statement

There were no conflicts of interest in carrying out this work.

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